FEEDBACK-RESISTANT PYRUVATE CARBOXYLASE GENE FROM CORYNEBACTERIUM

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CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of the filing date of U.S. Provisional Application No. 60/239,913, filed on October 13, 2000, which is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

Field of the Invention

[0001] The present invention relates to a mutated pyruvate carboxylase gene from *Corynebacterium*. The mutant pyruvate carboxylase gene encodes a pyruvate carboxylase enzyme which is resistant to feedback inhibition from aspartic acid. The present invention also relates to a method of replacing the wild-type pyruvate carboxylase gene in *Corynebacterium* with this feedback-resistant pyruvate carboxylase gene. The present invention further relates to methods of the production of amino acids, preferably lysine, comprising the use of this mutant pyruvate carboxylase enzyme in microorganisms.

Background Art

[0002] Pyruvate carboxylase is an important biotin-containing enzyme found in a variety of plants and animals, as well as some groups of bacteria (Modak, H.V. and Kelly, D.J., Microbiology 141:2619-2628 (1995)). In the presence of adenosine triphosphate (ATP) and magnesium ions, pyruvate carboxylase catalyzes the two-step carboxylation of pyruvate to form oxaloacetate, as shown in the equations below:

$$MgATP + HCO_3^- + ENZ\text{-}biotin \xrightarrow{Mg^{2+}\text{acetyl-CoA}} MgADP + Pi + ENZ\text{-}biotin\text{-}CO_2 \quad (1)$$

$$ENZ\text{-}biotin\text{-}CO_2 + Pyruvate \longrightarrow ENZ\text{-}biotin + oxaloacetate \quad (2)$$

[0003] In reaction (1) the ATP-dependent biotin carboxylase domain carboxylates a biotin prosthetic group linked to a specific lysine residue in the biotin-carboxyl-carrier protein (BCCP) domain. Acetyl-coenzyme A activates reaction (1) by increasing the rate of bicarbonate-dependent ATP cleavage. In reaction (2), the BCCP domain donates the CO_2 to pyruvate in a reaction catalyzed by the transcarboxylase domain (Attwood, P.V., *Int. J. Biochem. Cell. Biol. 27*:231-249 (1995)).

[0004] In bacteria such as *Corynebacterium glutamicum*, pyruvate carboxylase is utilized during carbohydrate metabolism to form oxaloacetate, which is in turn used in the biosynthesis of amino acids, particularly L-lysine and L-glutamate. Furthermore, in response to a cell's metabolic needs and internal environment, the activity of pyruvate carboxylase is subject to both positive and negative feedback mechanisms, where the enzyme is activated by acetyl-CoA, and inhibited by aspartic acid. Based on its role in the pathway of amino acid synthesis, and its ability to be regulated, pyruvate carboxylase plays a vital role in the synthesis of amino acids.

[0005] Bacteria such as *C. glutamicum* and *E. coli* are widely used in industry for the production of amino acids such as L-glutamate and L-lysine. Because of the central importance of pyruvate carboxylase in the production of amino acids, particularly L-glutamate and L-lysine, the exploitation of pyruvate carboxylase to increase amino acid production is of great interest in an industrial setting. Thus, promoting the positive feedback mechanism of pyruvate carboxylase, or

inhibiting its negative feedback mechanism, in *C. glutamicum* or could augment amino acid production on an industrial scale.

BRIEF SUMMARY OF THE INVENTION

[0006] One aspect of the present invention relates to a nucleic acid molecule comprising a nucleotide sequence which codes for a pyruvate carboxylase of SEQ ID NO:19, wherein this pyruvate carboxylase contains at least one mutation which desensitizes the pyruvate carboxylase to feedback inhibition by aspartic acid.

[0007] Another aspect of the present invention provides methods for using the nucleic acid of SEQ ID NO:1 or SEQ ID NO:3, which encodes the amino acid sequence of a mutant pyruvate carboxylase. Such uses include the replacement of the wild-type pyruvate carboxylase with the feedback-resistant pyruvate carboxylase, and the production of amino acids. An additional aspect of the present invention provides a polypeptide comprising the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4. Still another aspect of the present invention provides a polypeptide comprising the amino acid sequence selected from the group comprising SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 SEQ ID NO:14, SEQ ID NO:16 and SEQ ID NO:18.

[0008] Another aspect of the present invention also relates to a nucleic acid molecule comprising a nucleotide sequence which encodes the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, or the amino acid sequence encoded by the DNA contained in Deposit Number NRRL B-11474. Another aspect of the present invention further relates to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1 and SEQ ID NO:3.